Claims
(cleaned up)

1. A method to generate both a cell-based and humoral immune response to a target polypeptide in an animal, which method comprises administering subcutaneously or intramuscularly to the animal a composition comprising liposomes suspended in an aqueous liquid and a polynucleotide comprising a promoter operatively linked to a nucleotide sequence encoding said target polypeptide,

wherein the liposomes have diameters in the range 100 to 2000 nm and comprise a lipid bilayer and an aqueous intravesicular space, wherein said polynucleotide is entrapped in the aqueous intravesicular space,

wherein said lipid bilayer includes at least one cationically charged component in an amount such that the lipid bilayer has an overall cationic charge;

whereby said polynucleotide is delivered to and is expressed in target cells whereby an immune response including an IgG response and Th1 and Th2 responses to the target polypeptide result;

wherein said polynucleotide is administered in an amount sufficient to elicit said immune response.

6. The method of claim 1, wherein said composition has been prepared by a process that comprises

mixing an aqueous suspension of empty liposomes with said polynucleotide to form a mixed suspension,

dehydrating the mixed suspension to form a dehydrated mixture, and

rehydrating the dehydrated mixture in an aqueous liquid to form liposomes which are dehydration - rehydration vesicles (DRVs) containing the polynucleotide in the intravesicular space.

7. The method of claim 6, wherein said process further includes subjecting said polynucleotide-containing DRVs to microfluidization or extrusion.

Exhibit A

8. The method of claim 6, wherein in said process said dehydrating is by lyophilizing.

11. The method of claim 1, wherein the cationic component is selected from the group consisting of DOTAP, BisHOP, DC-Chol and stearylamine.

12. The method of claim 1, wherein the lipid bilayer includes a phosphatidyl ethanolamine.

13. The method of claim 1, wherein the mean diameter of the liposomes is in the range of 200 to 500 nm.

14. The method of claim 1, wherein said composition comprises 0.1 to 10 μg of polynucleotide per mg lipid bilayer.

16. The method of claim 1, wherein the composition is administered intramuscularly.

17. A process for forming an aqueous suspension of liposomes having diameters in the range 100 to 2000 nm comprising the steps:

a) providing an aqueous suspension of small unilamellar vesicles formed from liposome-forming agents selected from the group consisting of lipids, cholesterol and non-ionic and cationic surface active agents, wherein at least one cationically charged component selected from cationic lipids and cationic surface active agents is present in an amount whereby the small unilamellar vesicles have an overall cationic charge;

b) adding to the aqueous suspension of small unilamellar vesicles a nucleic acid including a promoter operatively linked to a nucleotide sequence encoding an immunogenic polypeptide to form a mixed suspension in which the weight ratio of liposome forming components making up the small unilamellar vesicles in step (a) to the nucleic acid added in step (b) is in the range (50 to 10000):1;

c) dehydrating the mixed suspension to form a dehydrated mixture;

Exhibit A

d) rehydrating the dehydrated mixture to form an aqueous suspension of liposomes that are dehydration-rehydration vesicles (DRVs) containing said nucleic acid in an intravesicular space thereof; and

e) optionally subjecting the aqueous suspension of DRVs to microfluidisation whereby said aqueous suspension of liposomes is produced.

18. The process of claim 17 further comprising removing non-entrapped nucleic acid from the aqueous suspension of DRVs.

19. The process of claim 18 wherein the level of non-entrapped nucleic acid separated from the suspension is in the range 10 to 90% based on polynucleotide added in step (b).

20. The process of claim 19 wherein said level is in the range 15 to 80%.

28. The process of claim 17, wherein the cationic component is selected from the group consisting of DOTAP, BisHOP, DC-Chol and stearylamine.

29. The process of claim 17, wherein the liposome forming components include a phosphatidyl ethanolamine.

30. The process of claim 17, wherein the small unilamellar vesicles in step (a) have a diameter in the range 100 to 400 nm.

31. The process of claim 17, wherein the dehydration-rehydration vesicles produced in step d) have diameters in the range 200 to 2000 nm.

Exhibit A

34. A composition for administration to an animal to induce a cell-based and humoral immune response to a polypeptide, which composition comprises

liposomes having diameters in the range 100 to 2000 nm and having lipid-bilayers surrounding aqueous intravesicular spaces and a polynucleotide comprising a promoter operatively linked to a nucleotide sequence encoding said target polypeptide,

which lipid-bilayers are formed from liposome forming components that include at least one cationic component in an amount to confer an overall cationic charge on the liposome forming components, and

wherein the polynucleotide is entrapped in the aqueous intravesicular space.

35. The composition of claim 39, wherein the viral polypeptide is a polypeptide of hepatitis B, hepatitis C, influenza or human immunodeficiency virus.

36. The composition of claim 35, wherein the viral polypeptide is hepatitis B surface antigen or haemagglutinin.

37. The composition of claim 34, wherein the liposomes are suspended in a pharmaceutically acceptable aqueous vehicle.

39. The composition of claim 34, wherein the polypeptide is a viral polypeptide.

Exhibit A